IN THE UNITED STATES DISTRICT COURT
FOR THE DISTRICT OF DELAWARE

AVENTIS PHARMA S.A., SANOFI-AVENTIS U.S., LLC	)	
Plaintiffs,	)	Civil Action No. 07-721-GMS (Consolidated)
v.	)	
	)	**REDACTED PUBLIC VERSION**
HOSPIRA, INC., APOTEX INC., and APOTEX CORP	)	
Defendants.	)	

PLAINTIFFS' ANSWERING BRIEF IN OPPOSITION TO HOSPIRA'S SECOND MOTION IN LIMINE: TO PRECLUDE SANOFI'S EXPERTS FROM TESTIFYING ABOUT THE CLAIM CONSTRUCTION OF PERFUSION

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Plaintiffs Aventis Pharma S.A. and sanofi-aventis U.S., LLC (collectively, "Plaintiffs" or "sanofi-aventis") respectfully submit this brief in opposition to Hospira, Inc.'s ("Hospira") Second Motion *in Limine*: To Preclude Sanofi's Experts from Testifying About the Claim Construction of Perfusion (D.I. 272) ("Hospira Mot.").

Hospira seeks to undo its stipulated agreement regarding the construction of the term "perfusion." As Hospira acknowledges in its motion, perfusions are pharmaceutical compositions administered intravenously to patients through an i.v. bag system (that is, they are the traditional formulation through which cancer patients receive chemotherapy). The parties *agreed* to the construction of perfusion as "a solution **suitable for infusion into patients** including at least active pharmaceutical ingredient and an aqueous infusion fluid such as physiological saline or glucose (emphasis added)." (D.I. 44 at 3.) Hospira now seeks to preclude Plaintiff's experts from testifying about application of a claim construction to which it admittedly agreed. (*See* Hospira Mot. at 3.) Hospira's motion should be denied for at least two independent reasons: *first,* because it is Hospira, not Plaintiffs, seeking to distort the meaning of "perfusion" and *second,* because Hospira's attempt to preclude expert testimony concerning application of the agreed-upon construction of the claim is improper and an improper use of the *in limine* process. Either reason suffices to defeat Hospira's motion.

Hospira asserts that sanofi's experts have inappropriately taken into account issues of toxicity, efficacy, and stability in determining whether a formulation is "suitable for infusion to patients," as the agreed claim construction requires.

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The construction Hospira proposes is extraordinary and extreme;

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Thus, in the guise of an *in limine* motion, Hospira is actually asking this Court to redefine the claim term "perfusion" to be any liquid that can be shot out of a needle, regardless of whether it is capable of being used therapeutically -- indeed, even if its administration would kill the patient. Hospira's proposal is clearly at odds with any rational understanding of the phrase "suitable for infusion into patients."

The reason for Hospira's attempt to rewrite the claim is transparent: it cannot succeed in its validity challenge to the claimed pharmaceutical compositions without reading out of the claims any requirement that the perfusion actually be suitable for infusion into patients. The development of perfusions—that is, of pharmaceutical formulations suitable for intravenous infusion into patients—is among the most challenging of tasks. By definition, the entire formulation is administered directly into the patient's blood stream, rendering issues of toxicity and stability of paramount importance. The courts, including this one, have repeatedly recognized that patent claims drawn to injectable formulations require considerations of biological activity and toxicity. *See, e.g., Amgen Inc. v. Hoechst Marion Roussel, Inc.*, 579 F. Supp. 2d 199, 205 (D. Mass. 2008) (construing "pharmaceutical composition" to mean "suitable for administration to humans" and finding prior art to be suitable for administration because it was "without any evidence of pyrogens or any significant deleterious consequences" and animal testing had shown no "adverse effects"); *Pharmacia & Upjohn Co. v. Sicor Inc.*, 447 F. Supp. 2d 363, 370 (D. Del. 2006) (construing the term "physiologically suitable" in the context of an injectable formulation to mean "sterile, pyrogen-free, and otherwise suitable for administration

to patients");[1] *see also Purdue Pharma, LP v. F.H. Faulding and Co.*, 48 F. Supp. 2d 420, 437 (D. Del. 1999) ("'effective treatment of pain' means that an individual patient is provided with adequate pain relief from the sustained release opioid dosage form without unacceptable side effects); *Abbott Labs. v. Sandoz, Inc.*, 529 F. Supp. 2d 893, 910-11 (N.D. Ill. 2007) ("'pharmaceutically acceptable polymer' . . . means any polymer, which within the scope of sound medical judgment is suitable for use in pharmaceutical compositions for use in contact with the tissues of humans and lower animals without undue toxicity, irritation, allergic response and the like, in keeping with a reasonable benefit/risk ratio, and effective for their intended end use in the chemotherapy and prophylaxis of antimicrobial infections and that extends drug release into the bloodstream").[2]

In short, the challenge in developing a perfusion is not, as Hospira would have it, merely getting the formulation out of the needle, but rather getting it safely and effectively into the patient. That is the plain and ordinary meaning of the phrase "suitable for infusion into patients" that both parties agreed to in construing "perfusion."[3] Patent claims are to be

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[1] In *Pharmacia*, the court did not impose a stability limitation because there was no discussion of stability in the patent or prosecution history. By contrast, stability is repeatedly emphasized in the patent and prosecution history here. (*See, e.g.*, '561 patent, 2:43-45 ("The new perfusions are stable from a physical standpoint, that is to say no precipitation phenomenon is seen to appear within approximately 8 hours"); *see also* 1:49-51; 3:14-15.)

[2] Hospira erroneously relies upon *Medeva Pharmaceuticals Mfg., Inc. v. Morton Grove Pharmaceuticals*, 174 F. Supp. 2d 802 (N.D.Ill. 2001). But *Medeva* involved a formulation "suitable for oral administration" not "suitable for infusion into patients." Whether the court there was correct that "drugs are routinely administered orally despite their being unstable, horrid-tasting, highly toxic and without therapeutic activity," such a statement would plainly be incorrect with regard to intravenously administered formulations like perfusions: no responsible clinician routinely administers an intravenous infusion that is unstable or highly toxic or without therapeutic activity. It is also noteworthy that, in the eight years since *Medeva* was decided, no other court has followed it.

[3] Both the '512 and the '561 patents reference the clinical problems associated with administering perfusions and both cite, for purposes of defining perfusions, to a 1990 article by (continued...)

understood in light of the problem to be solved. *See, e.g., Eastman Kodak Co. v. Goodyear Tire & Rubber Co.*, 114 F.3d 1547, 1554 (Fed. Cir. 1997), *abrogated on other grounds by Cybor Corp. v. Fas Techs. Inc.*, 138 F.3d 1448 (Fed. Cir. 1998) ("The specification, of which the claims are part, teaches about the problems solved by the claimed invention, the way the claimed invention solves those problems, and the prior art that relates to the invention. These teachings provide valuable context for the meaning of the claim language."). It is Hospira, not Plaintiffs, that now seeks to depart from this understanding.

Independently, Hospira's motion should be denied because it is an inappropriate attempt to relitigate claim construction through an *in limine* motion. In *Corning, Inc. v. SRU Biosystems*, No. 03-633, 2005 WL 2465900, *2 (D. Del. Oct 5, 2005), this Court denied just such a motion *in limine* to preclude argument or testimony asserted to be contrary to the court's claim construction. This Court concluded that ample opportunity was afforded at trial to cross-examine the expert on his opinions to the extent that they purportedly departed from the Court's claim construction, and reasoned that any deviations between the expert's testimony and the Court's claim construction would be considered by the Court in determining the weight to be given to the expert's testimony. *Id.* This is the approach consistently taken by courts. *See, e.g.., 3COM Corp. v. Realtek Semiconductor Corp.*, No. 03-2177, 2008 WL 783383, at *4 (N.D. Cal. March 24, 2008) (denial of motion *in limine* to exclude expert opinion alleged to be based on claim definitions other than those established in Markman order); *Kemin Foods, L.C., v. Pigmentos Vegetales Del Centros S.A. de C.V.*, No. 02-40327, 2004 WL 550872, at *4 (S.D.

Rowinsky and coworkers and the National Cancer Institute that contains an extensive discussion of the safety and effectiveness issues associated with developing perfusions of the highly water-insoluble taxanes. (*See* Exhibit B at 1250-1255.)

Iowa Sept. 9, 2004) (same, and noting that parties may permissibly disagree about the application of the court's claim constructions).

With this motion Hospira is attempting to prevent the Court from having the opportunity even to hear Plaintiffs' experts' application of the agreed-upon construction of the term "perfusion" and then decide for itself whether it is Plaintiffs or Hospira who has properly understood and applied the requirement that it be "suitable for infusion into patients." Hospira's motion should be denied.

CONCLUSION

For the foregoing reasons, sanofi-aventis respectfully requests that the Court deny Hospira's Second Motion *in Limine*.

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EXHIBIT A

EXHIBIT REDACTED

EXHIBIT B

REVIEW

Taxol: A Novel Investigational Antimicrotubule Agent

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Microtubules are among the most strategic subcellular targets of anticancer chemotherapeutics. Despite this fact, new antimicrotubule agents that possess unique mechanisms of cytotoxic action and have broader antineoplastic spectra than the vinca alkaloids have not been introduced over the last several decades—until the recent development of taxol. Unlike classical antimicrotubule agents like colchicine and the vinca alkaloids, which induce depolymerization of microtubules, taxol induces tubulin polymerization and forms extremely stable and nonfunctional microtubules. Taxol has demonstrated broad activity in preclinical screening studies, and antineoplastic activity has been observed in several classically refractory tumors. These tumors include cisplatin-resistant ovarian carcinoma in phase II trials and malignant melanoma and non-small cell lung carcinoma in phase I studies. Taxol's structural complexity has hampered the development of feasible processes for synthesis, and its extreme scarcity has limited the use of a conventional, broad-scale screening approach for evaluation of clinical antitumor activity. However, taxol's unique mechanism of action, its spectrum of preclinical antitumor activity, and tumor responses in early clinical trials have generated renewed interest in pursuing its development. [J Natl Cancer Inst 82:1247–1259, 1990]

Antimicrotubule agents are among the most important anticancer drugs and have significantly contributed to the therapy of most curable neoplasms, such as Hodgkin's and non-Hodgkin's lymphomas, germ cell tumors, and childhood leukemia. These agents have also been used in the palliative treatment of many other cancers. On a subcellular level, they are extremely potent, as exemplified by the ability of only a few molecules to disrupt the microtubular substructure of cancer cells (*1*). Despite their promise, only a few antimicrotubule agents have been developed over the last few decades. None have clearly possessed novel mechanisms of cytotoxic action, and none have demonstrated broader antitumor spectra or more advantageous therapeutic indices than the vinca alkaloids until, perhaps, the recent development of taxol.

All antimicrotubule agents used clinically are structurally complex natural products; the vinca alkaloids and colchicine are derived from the periwinkle and meadow saffron plants, respectively. Taxol (fig. 1) is the first compound with a taxene ring that has been demonstrated to possess antineoplastic activity, and it has become one of the most important lead anticancer agents to recently emerge from the screening of natural products.

Interest in taxol dates from the late 1960s, when a crude extract of bark from the Pacific yew *Taxus brevifolia* was tested by the National Cancer Institute (NCI) in a large-scale screening program. In the test, the extract demonstrated cytotoxic activity against several murine tumors, including L1210, P388, and P1534 leukemias, Walker 256 carcinosarcoma, sarcoma 180, and Lewis lung tumors (2).

In 1971, Wall and co-workers at the Research Triangle Institute (Durham, NC) (2) identified taxol as the active constituent of the bark extract. Despite promising antitumor activities, development of taxol as an antineoplastic agent has progressed slowly, primarily because of its scarcity and the difficulty of large-scale isolation, extraction, and preparation. In addition, taxol's poor aqueous solubility has hampered the development of a suitable clinical formulation.

Interest in taxol was later rekindled by two factors. First, taxol's unique mechanism of cytotoxic action as a promoter of microtubule assembly was discovered (*3,4*), and second, a determined effort on the part of the NCI resulted in adequate drug supplies for conventional preclinical screening, toxicological studies, and preliminary clinical trials.

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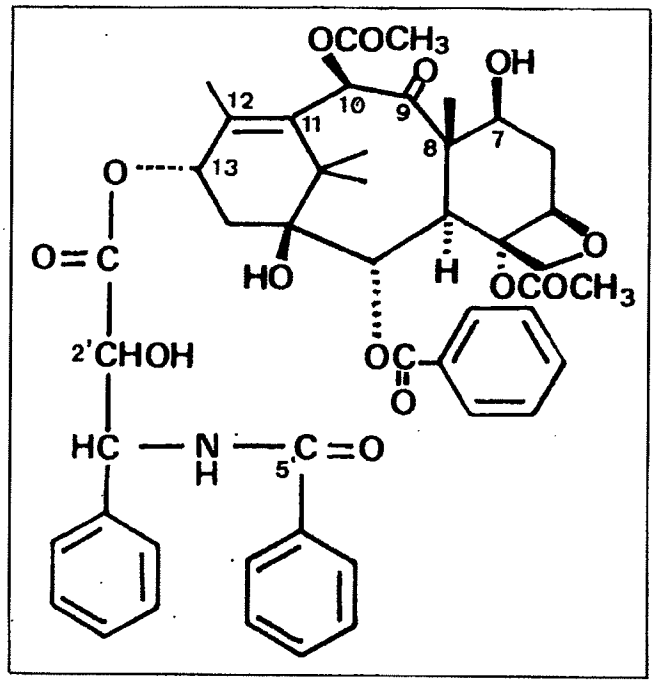

Figure 1. Structure of taxol.

Preclinical Studies

Mechanism of Action

Like DNA and RNA, microtubules are ubiquitous cellular constituents (*5*). Although microtubules are commonly believed to be important only in mitotic functions as primary constituents of the mitotic spindle apparatus, they are also critical for the performance of many vital interphase functions, such as maintenance of cell shape, cellular motility and attachment, and intracellular transport. They may also play a key role in modulating (*a*) the interactions of growth factors with cell-surface receptors and (*b*) the proliferative transmembrane signals generated by these interactions (*5–14*). Microtubules are in a dynamic equilibrium with their basic protein subunits, which are tubulin dimers (*5,15*). The direction of this equilibrium toward microtubule assembly or disassembly appears to be determined by signals generated during specific cell cycle phases by intracellular regulators like calcium and guanosine triphosphate (GTP) (*6,15*).

The molecular pharmacology of taxol was largely elucidated by Horwitz and co-workers at the Albert Einstein College of Medicine (New York, NY) (*3,4,15–19*). These investigators demonstrated that taxol binds preferentially to microtubules rather than to tubulin dimers, with a stoichiometry approaching 1 mole of taxol per 1 mole of polymerized tubulin dimer (*16*). The binding of taxol to polymerized tubulin is reversible, and the binding constant is approximately 0.9 μmol/L (*16,20*). The investigators also determined that the binding site for taxol on microtubules was distinct from the binding sites for exchangeable GTP and for colchicine, podophyllotoxin, and vinblastine (*19,21*).

Unlike other antimicrotubule agents that induce microtubule disassembly, such as colchicine and the vinca alkaloids, taxol promotes microtubule assembly and stabilizes microtubules by shifting the dynamic equilibrium toward microtubule assembly (*3,4,15–27*). Taxol concentrations as low as 0.05 μmol/L promote microtubule assembly in vitro, decrease the lag time for microtubule assembly, and shift the equilibrium in favor of microtubule formation (*3*). In essence, taxol decreases the critical concentration of tubulin required for microtubule assembly in the presence or absence of factors that are usually essential for this function, such as exogenous GTP or microtubule-associated proteins (*19,28*). Microtubules treated with taxol are stable even after treatment with calcium or low temperatures, conditions that usually promote disassembly (*3,4,29*). This unusual stability results in the inhibition of the normal dynamic reorganization of the microtubule network.

Clinically relevant concentrations of taxol (0.1–10.0 μmol/L) produce two distinct morphological effects on microtubules in cells, as assessed by electron microscopy and indirect immunofluorescence (figs. 2A, 2B, and 2C). First, cells treated with taxol form abundant arrays of disorganized microtubules that are often aligned in parallel bundles (fig. 2B) (*4,15,30–35*). These bundles form during all phases of the cell cycle. Taxol also induces the formation of abnormal spindle asters during mitosis (fig. 2C) (*15,35–41*). Under normal conditions during mitosis, centrioles migrate to opposite poles of the cell, each forming a spindle aster that aids in separating duplicated chromosomal material before cell division. In contrast, mitotic cells treated with taxol form excessive numbers of abnormal asters that do not require centrioles for enucleation and are reversible following treatment (*35,39*).

Early studies with HeLa cells and BALB/c mouse fibroblasts treated with low concentrations of taxol (0.25 μmol/L), which produce minimal inhibition of DNA, RNA, and protein synthesis (*3*), demonstrated that taxol blocks cell cycle traverse in the mitotic phases (*4,42*). Studies of murine P388 leukemia showed similar findings. More recently, taxol has been demonstrated to prevent transition from the G_0 phase to the S phase in fibroblasts during stimulation of DNA synthesis by growth factors and to delay traverse of sensitive leukemia cells in nonmitotic phases of the cell cycle (*6,35*). These findings indicate that the integrity of microtubules may be critical in the transmission of proliferative signals from cell-surface receptors to the nucleus (*6–14*).

Proposed explanations that at least in part account for taxol's inhibitory effects in nonmitotic phases include disruption of

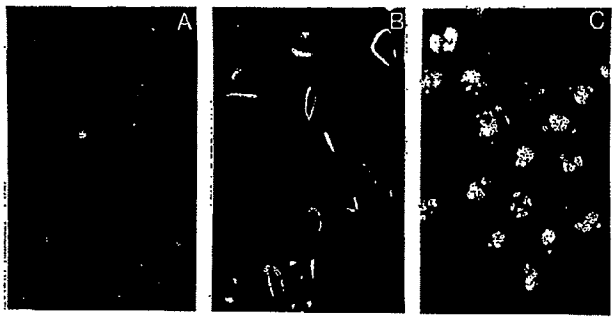

Figure 2. Indirect immunofluorescence staining of taxol-induced microtubule effects in leukemia cell lines. A: untreated K562 cells; B: microtubule bundles in taxol-treated HL-60 cells; and C: taxol-induced mitotic spindle asters in K562 cells.

tubulin in the cell membrane (*35,43,44*) and/or direct inhibition of the disassembly of the interphase cytoskeleton, which may upset many vital cell functions such as locomotion (*45*), intracellular transport (*46*), and transmission of proliferative transmembrane signals (*6–14*). Indeed, in Walker 256 carcinosarcoma cells, taxol has been demonstrated to inhibit shape changes and locomotion, an effect that may be related to the invasiveness and metastatic potential of tumors (*45*). Taxol has also been shown to inhibit steroidogenesis in human Y-1 adrenocortical tumors and in MLTC-1 Leydig tumors by decreasing the intracellular transport of cholesterol to cholesterol side-chain cleavage enzymes (*46*). These effects on locomotion, shape changes, and intracellular transport appear to be related to perturbations in microtubule dynamics (*45,46*).

Taxol has also been shown to inhibit specific functions in many nonmalignant tissues, which may be mediated through microtubule disruption. For example, in human neutrophils, taxol inhibits its relevant morphological and biochemical processes, including chemotaxis, migration, cell spreading, polarization, generation of hydrogen peroxide, and killing of phagocytosed microorganisms (*47,48*). Taxol also antagonizes the effects of microtubule-disrupting drugs on lymphocyte function and adenosine 3′,5′-cyclic monophosphate metabolism and inhibits the proliferation of stimulated human lymphocytes, but blast transformation is not affected during lymphocyte activation (*49,50*). Taxol has also been found to mimic the effects of endotoxic bacterial lipopolysaccharide on macrophages, resulting in a rapid decrement of receptors for tumor factor-α and tumor necrosis factor-α release (*51*). This finding suggests that an intracellular target affected by taxol may be involved in the actions of lipopolysaccharide on macrophages and other cells. Interestingly, taxol inhibited chorioretinal fibroblast proliferation and contractility in an in vitro model of proliferative vitreoretinopathy, a fact that may be relevant to the treatment of traction retinal detachment and proliferative vitreoretinopathy (*52*). Finally, taxol has been shown to inhibit the secretory functions of many specialized cells. Examples include insulin secretion in isolated rat islets of Langerhans (*53*), protein secretion in rat hepatocytes (*54,55*), and the nicotinic receptor-stimulated release of catecholamines from chromaffin cells of the adrenal medulla (*56,57*).

Elaborate structure–activity studies have demonstrated that the substituent on the carbon in the 13-position of taxol's taxene ring is absolutely required for cytotoxicity, as well as to promote microtubule assembly in the absence of GTP (*58–67*). Although acetylation of taxol at the 2′-position does not attenuate cytotoxic activity, it does not promote microtubule assembly in a cell-free system. This discrepancy suggests that taxol undergoes deacylation in living cells. However, congeners that lack an accessible 2′-hydroxyl group are much less active than the parent compound, which indicates that a free 2′-hydroxyl group is required for maximal activity.

In an attempt to increase the agent's solubility and develop more feasible clinical formulations, investigators have acylated carbons of taxol's taxene ring at the 7-position and 10-position. These efforts have yielded compounds that retain both cytotoxic and microtubule-promoting activities. In the future, similar studies may be critical for identifying related natural products and for the synthesis of derivatives that have greater activity and aqueous solubility than taxol.

Mechanisms of Resistance

Investigations with cell lines made resistant to taxol have characterized two potential mechanisms of acquired drug resistance. First, some Chinese hamster ovary cell lines with acquired taxol resistance possess altered α-tubulin or β-tubulin and require taxol in the medium for normal growth (*67–69*). Studies using indirect immunofluorescence and electron microscopy have demonstrated that these resistant cells lack normal microtubules in their interpolar mitotic spindles when grown in the absence of taxol. In essence, these cells have mutations in tubulin, resulting in impaired microtubule assembly. Continuous exposure to taxol is required for polymerization to proceed normally, thereby promoting the formation of functional microtubules.

A second well-documented mechanism of acquired taxol resistance involves the multidrug-resistance phenotype that confers varying degrees of cross-resistance to other antimicrotubule agents and natural products, including colchicine, taxol, vincristine, doxorubicin, and etoposide (*70–77*). The multidrug phenotype, which has been characterized extensively in human and murine cell lines, is associated with genomic alterations resulting in the overexpression of phosphorylated membrane glycoproteins, which function as drug efflux pumps. Roy, Horwitz, and associates (*75,76*) have also described a unique phosphoglycoprotein with a relative molecular mass (M_r) of 135,000 that accounts for 4%–5% of the total membrane protein of a murine macrophage cell line (J774.2) that is 800 times more resistant to taxol than its parent line. This glycoprotein is similar, but not identical, to the one found in vinblastine- and colchicine-resistant cells derived from the same parental line. In the continuous presence of taxol, these taxol-resistant cells accumulate less drug at steady state than sensitive parental cells. They are also cross-resistant to colchicine, vinblastine, dactinomycin, and doxorubicin, but sensitive to bleomycin. However, they become more sensitive to taxol and develop progressive decrements in the M_r 135,000 membrane glycoprotein as durations in drug-free medium are prolonged.

Additionally, Brewer and Warr (*77*) have described two groups of Chinese hamster ovary lines with low resistance to vincristine that may shed light on mechanisms of acquired taxol resistance. These two groups include (*a*) cell lines that were cross-resistant to taxol and that were thought to be membrane mutations, because of their decreased ability to accumulate vincristine and their reversal of resistance with calcium-channel blockers, and (*b*) cell lines that were sensitive to taxol and that were thought to have tubulin mutations (*77*).

Although the precise mechanisms that account for inherent resistance to taxol have not been clearly defined, several interesting observations have been made in human leukemia cell lines (*35,78,79*). The sensitivity of these lines to taxol was directly related to the taxol-induced formation of irreversible microtubule bundles (*35*). While sensitive cell lines primarily formed irreversible bundles and were critically affected during interphase, most relatively resistant cells were unaffected during traverse through G_0/G_1 and S phases. The resistant cells accumulated in the G_2/M phase and formed multiple abnormal asters (*35*). Most resistant cells that formed asters also contained polyploid DNA after prolonged drug exposure. The magnitude of DNA polyploidization appeared to be related to resistance (*79*). Interestingly, in a

phase I trial of taxol in leukemia, the sensitivity of leukemic blasts to form microtubule bundles was directly related to the magnitude of clinical antitumor activity (*80*). These studies suggest that microtubule bundles and DNA polyploidization may be useful markers of lethal drug effects that could be detected in biopsy specimens and evaluated in prospective clinical trials.

Antineoplastic Activity

In 1971, taxol was reported to be cytotoxic in the KB cell culture system and to be moderately active against murine L1210, P388, and P1534 leukemias, the Walker 256 carcinosarcoma, sarcoma 180, and Lewis lung tumor. Nevertheless, it was not selected for clinical development by the NCI until 1977 (*2,81*). At that time, the decision to develop taxol was based on new knowledge about the agent's unique mechanism of antineoplastic action and impressive activity against the murine B16 melanoma, a tumor incorporated into the tumor panel of the Division of Cancer Treatment (DCT) in 1975 (*81*).

Taxol also demonstrated significant antitumor activity against several xenografts of human tumors in the DCT screening tumor panel of the late 1970s, including the MX-1 mammary tumor implanted beneath the renal capsule of athymic mice. When the drug was administered subcutaneously, it showed moderate activity against similar xenografts of human CX-1 colon and LX-1 lung tumors (*81*) and against interperitoneally implanted P388 and L1210 murine leukemias. However, the drug proved ineffective against the murine $CD8F_1$ mammary and colon 38 carcinomas that were subcutaneously implanted and the intravenously implanted Lewis lung carcinoma. Investigations of schedule dependency in the P388 leukemia system revealed that increased life span was maximal when mice were treated every 3 hours, which is functionally equivalent to continuous administration (*81*).

Taxol has also demonstrated notable cytotoxic activity against several human leukemias and solid tumors in early studies performed by many independent investigators. Riondel, Jacrot, and associates (*82,83*) reported that taxol induced substantial regression in four of five human breast carcinoma implants in nude mice and significant growth delays in xenografts of human endometrial, ovarian, brain, tongue, and lung tumors (*82,83*). These investigators also studied the effects of taxol and a congener with a substituent variation on the carbon in the 13-position. They demonstrated that taxol produced significant cytotoxic activity in a human ovarian adenocarcinoma xenograft in nude mice, but the derivative was inactive (*84*). In contrast, taxol was inactive against several subcutaneous implants of human pancreatic carcinoma in nude mice (*85*). In addition, treatment of the human prostatic adenocarcinoma cell line LNCaP with taxol concentrations as low as 10 nmol/L for 9 days induced cytotoxicity and obvious lethal effects, as assessed by light microscopy. Lower concentrations (1 nmol/L), however, induced changes that were detected only by electron microscopy (*86*).

Other studies focused on developing more efficient screening systems for new antineoplastic agents. Fan et al. (*87*) compared the antitumor activities of several standard and investigational antitumor agents at equivalent inhibitory concentrations of granulocyte-macrophage colony-forming units (GM-CFUs) in a primary human tumor culture assay. Clinically active standard agents tested at concentrations up to their GM-CFU IC_{90} (concentration that inhibits 90% of growth) produced at least 50% inhibition of growth in at least 30% of human tumors. However, tumor responses with the investigational agents taxol, caracemide, and spirogermanium were 78%, 9% and 7%, respectively. On the basis of this model and knowledge that myelosuppression was emerging as taxol's principal clinical toxic effect in concurrent phase I studies, Fan et al. predicted that taxol would be an active as well as clinically efficacious anti-neoplastic agent.

Micromolar concentrations of taxol have also been demonstrated to induce unique morphological changes and to block the replication of *Trypanosoma cruzi*, the causative agent of South American trypanosomiasis. No curative therapy is known for this disease (*88*); therefore, taxol may have potential as a protozoacide.

Preclinical Toxicology

The preclinical toxicology of taxol has been studied in $CD2F_1$ mice, Sprague-Dawley rats, and beagle dogs (*81*). In mice and rats, the interperitoneal route of administration was used because of dose-volume constraints that were imposed by taxol's limited aqueous solubility and the toxicity of its cremophor vehicle. In lethality studies, rats were treated once a day for 1 day or once a day for 5 days, while mice were treated once a day for 5 days. Doses lethal to 10%, 50%, and 90% (LD_{10}, LD_{50}, and LD_{90}, respectively) of the rats given a single dose were 138, 206, and 307 mg/m^2, respectively. The LD_{10}, LD_{50}, and LD_{90} for rats treated once a day for 5 days were 36, 51, and 74 mg/m^2 per day, respectively, whereas corresponding values in mice were 70, 82, and 97 mg/m^2 per day. Toxicity was also evaluated for both schedules in beagle dogs that were given taxol intravenously.

The toxic effects of taxol were most evident in tissues with rapid cell turnover, including hematopoietic, lymphatic, gastrointestinal, and reproductive tissues. Hematopoietic toxic effects were observed in all three species, but the differential toxicity in hematopoietic tissues varied among species. In beagle dogs, myelosuppression was cumulative and reversible between 45 and 180 mg/m^2 on a single-dose schedule, and between 15 and 30 mg/m^2 per day on a schedule of five daily doses. Dose-related lymphoid depletion was also observed in all species. Gastrointestinal toxic effects were more severe in dogs receiving five daily doses and included dose-related weight loss, diarrhea, emesis, adipsia, and mucosal ulcerations. Pathologic changes such as diffuse inflammation and congestion of both the small and large intestines occurred in dogs at lethal doses. Testicular lesions characterized by necrosis of developing spermatocytes, giant cell formation in the seminiferous tubules, and oligospermia were also observed in rodents at both LD_{10} and LD_{50}. The reversibility of these lesions was not assessed. Effects on nerve, hepatic, cardiovascular, and renal tissues were minor, with no postmortem evidence of end-organ damage.

The vehicle in which taxol was formulated has been shown to have inherent toxicity. Cremophor EL (polyoxyethylated castor oil), given in increasing doses as a large-volume single dose without taxol, produced vasodilation, labored breathing, lethargy, hypotension, and death in dogs. The vehicle was much better tolerated in repeated doses and did not have cumulative toxicity. Although dogs were previously reported to be extremely

sensitive to the hypotensive effects of the cremophor component of the vehicle (*89*), these results suggest that clinical studies should be approached cautiously.

Preclinical Pharmacology

Taxol's aqueous insolubility hampered the development of a suitable analytical assay, which resulted in a lack of detailed pharmacologic information when taxol entered clinical trials. When available, such data have often been used to design rational schedules for clinical evaluation. Although several assays had previously been published (*59,90*), they were generally tedious and not applicable to clinical trials. Hamel et al. (*90*) described a unique biochemical assay with a sensitivity of 0.1 μmol/L. This assay exploited the ability of taxol to induce tubulin to form cold-resistant polymers that hydrolyze GTP at 0 °C. The investigators found that taxol was almost entirely bound to plasma proteins (92%) in rabbits, but nevertheless, was rapidly cleared from serum. In addition, the alpha and beta elimination half-lives of taxol administered as a rapid intravenous bolus to a single rabbit were 2.7 and 45.0 minutes, respectively (*90*).

Pharmaceutical Data

Taxol is supplied by the NCI as a concentrated sterile solution of 6 mg/mL in 5-mL ampules (30 mg per ampule). Because of taxol's aqueous insolubility, it is formulated in 50% cremophor EL and 50% dehydrated alcohol. The contents of the ampule must be diluted further in either 0.9% sodium chloride or 5% dextrose (*81*). During early phase I and II studies, taxol was diluted to final concentrations of 0.03–0.60 mg/mL. These concentrations were demonstrated to be stable for 24 and 3 hours, respectively, in early stability studies (*81*). This short stability period required the administration of large volumes of fluids and/or drug preparation at frequent intervals for patients receiving higher doses. In recent studies (*91,92*), concentrations of 0.3–1.2 mg/mL in either 5% dextrose or normal saline solution have demonstrated both chemical and physical stability for at least 12 hours. Only glass or polyolefin containers and polyethylene-lined nitroglycerin tubing have been recommended for drug administration since significant amounts of the plasticizer diethylhexylphthalate are leached from plastic tubing and solution bags containing polyvinyl chloride after contact with cremophor (*91*).

Clinical Trials

Phase I

Phase I trials of many schedules of taxol were begun in 1983 under the auspices of the DCT, but a high incidence of acute hypersensitivity reactions led to the premature discontinuation of many trials and threatened the prospects of taxol's further development. Initially, these reactions (*see* Toxic Effects section) appeared to resemble hypersensitivity reactions. They also occurred more frequently when taxol was infused over shorter durations. Therefore, infusion durations were increased to 6 or 24 hours and "anti-allergic" regimens consisting of steroids and H_1- and H_2-histamine antagonists were used prophylactically. Table 1 lists all completed and ongoing phase I studies, principal toxic effects, and doses recommended for phase II studies and observed maximum tolerated doses.

Neutropenia was the major dose-limiting toxic effect in all phase I solid tumor trials (*92–98*), except for a study conducted at Memorial Sloan-Kettering Cancer Center (New York, NY) in

Table 1. Phase I studies of taxol

Institution* (Ref. No.)	Schedule	Maximum tolerated dose (mg/m^2)	Recommended phase II dose (mg/m^2)	Dose-limiting toxic effect	Other principal effects
JHOC† (*93*)	1-6 hr infusion every 21 days	265	210	Neutropenia	Neuropathy, mucositis, arthralgias/myalgias, hypersensitivity reactions
Einstein† (*94*)	1- to 6-hr infusion every 21 days	275	250	Neutropenia Neuropathy	Hypersensitivity reactions, alopecia, mucositis
Einstein (*95*)	24-hr infusion every 21 days	275	250	Neutropenia Neuropathy	Hypersensitivity reactions
Memorial (*99*)	3-hr infusion every 21 days	190		Hypersensitivity reactions	Leukopenia, nausea, alopecia
UTSA (*92*)	6-hr infusion every 21 days	275	225	Neutropenia	Arthralgias/myalgias, mucositis, alopecia, neuropathy, hypersensitivity reactions
Mt. Sinai (*98*)	24-hr infusion every 21 days	200		Neutropenia	Alopecia, nausea, vomiting
MDA (*96*)	1-hr infusion × 5 days every 21 days	40	20	Neutropenia	Alopecia, diarrhea
Wisconsin (*97*)	1- to 6-hr infusion × 5 days every 21 days	40	30	Neutropenia	Hypersensitivity reactions, nausea, vomiting, alopecia, mucositis, thrombocytopenia
JHOC (*80*)	24-hr infusion every 14–21 days	390	310	Mucositis	Neutropenia, hypersensitivity reactions, neuropathy
JHOC (*100*)	24-hr infusion plus cisplatin every 21 days	135–170 + 75 (cisplatin)	135–170 + 75 (cisplatin)	Neutropenia	Arthralgias/myalgias, alopecia, cardiac, hypersensitivity reactions, neuropathy

*JHOC = Johns Hopkins Oncology Center, Baltimore, Md; Einstein = Albert Einstein College of Medicine, New York, NY; Memorial = Memorial Sloan-Kettering Cancer Center, New York, NY; UTSA = The University of Texas, San Antonio; Mt. Sinai = Mount Sinai School of Medicine, New York, NY; MDA = The University of Texas M.D. Anderson Cancer Center, Houston; Wisconsin = University of Wisconsin-Madison.

†Infusion duration lengthened during study because of hypersensitivity reactions and premedications added (steroids, H_1- and H_2-antihistamines). Maximum tolerated dose, recommended phase II dose, and dose-limiting toxic effects reflect longest duration of infusion with premedications.

which frequent hypersensitivity reactions precluded further dose escalation (*99*). Investigators at the Albert Einstein School of Medicine were also impressed that both peripheral neurotoxicity and neutropenia were dose limiting with 6- and 24-hour infusions (*94,95*). However, neurotoxicity was not particularly prominent at comparable taxol doses in other phase I and II studies. In fact, mucositis was the nonhematologic dose-limiting toxic effect of taxol in leukemia patients treated with higher doses than those that induced severe neutropenia in patients with solid tumors (*80*).

It was anticipated that neurotoxicity might preclude treatment with combinations of taxol and other neurotoxic agents like cisplatin. However, signs and symptoms of neurotoxicity and neurometric evidence of neurological dysfunction, as assessed by changes in electromyography and nerve conduction velocity, have been insignificant in an ongoing phase I study of cisplatin plus taxol (*100*). Instead, neutropenia has been the principal dose-limiting toxic effect. Consequently, on the basis of data indicating that combination of taxol and cisplatin induces maximal cytotoxicity in L1210 leukemia when treatment with taxol precedes cisplatin (*101*). Consequently, this study also compared the maximum tolerated doses and evaluated the severity of relative toxic effects of alternate sequences of drug treatment. The trial demonstrated that both nadir neutrophil counts were lower and that incidence of grade 4 neutropenia (neutrophils $\leq$ 500 cells per μL) was higher when treatment with cisplatin preceded taxol treatment. This observation may be explained pharmacologically by noting that taxol's clearance at steady state was significantly lower when cisplatin preceded taxol, compared with the alternate sequence (Rowinsky EK: unpublished data).

In early phase I trials, antineoplastic activity has been observed in several tumor types, including melanoma (four partial responses), adenocarcinoma of unknown origin (two partial responses), refractory ovarian carcinoma (one partial response, one minor response), non-small cell lung carcinoma (three partial responses, one minor response), and gastric (one minor response), colon (one minor response), and head and neck (one minor response) carcinomas. Antineoplastic activity has also been demonstrated in lymphoblastic and myeloblastic leukemias (*80,92–99*) (Brown T: personal communication). To date, the combination of taxol and cisplatin has also induced a pathologically documented complete response in a patient with an advanced large cell lung carcinoma, and antineoplastic activity has been noted in other advanced carcinomas: non-small cell lung carcinoma (two partial responses, two minor responses); breast (one partial response, one minor response), head and neck (one partial response), and colon carcinomas (two minor responses); advanced ovarian carcinoma (one clinical complete response, three partial responses, one minor response); and melanoma (one minor response) (*100*) (Rowinsky EK: unpublished data).

Phase II

Initially, severe shortages in the supply of taxol precluded conventional, broad-scale phase II trials. Instead, limited phase II trials have been performed in specific neoplasms on the basis of antitumor activity observed in preclinical and early phase I studies. To date, phase II studies that used 24-hour administration schedules and prophylactic anti-allergic medications have been completed in melanoma and in renal cell and advanced ovarian carcinomas (*102–106*). In renal cell carcinomas, taxol appeared to have low activity, since no responses were observed in 18 patients treated with 250 mg/m^2 (*102*). Taxol has been noted to have some activity in melanoma. It was active in preclinical murine models and some responses were observed in phase I trials (*94,95*). Objective response rates of 12% (three partial responses in 25 patients and four minor responses) and 18% (two complete responses and three partial responses in 28 patients) were noted in preliminary reports of two phase II trials completed to date (*103,104*). A more precise estimate of the degree of activity will require additional trials and careful attention to a variety of prognostic factors, including sites of metastatic disease, performance status, and amount of prior therapy. Some of these issues will be addressed in studies using higher taxol doses in combination with colony-stimulating factors.

The most exciting antineoplastic activity that has been observed with taxol has clearly been in advanced ovarian epithelial neoplasms. McGuire et al. (*105*) observed 11 partial responses, one pathological complete response, and seven minor responses of relatively long durations in 40 assessable patients. Besides demonstrating an extraordinarily high response rate in patients with advanced disease (partial response + complete response = 30%; partial response + complete response + minor response = 40%), these results are particularly important for two other reasons. First, most patients, including responders, were heavily pretreated with radiation and chemotherapy; the mean number of chemotherapy regimens per patient was 2.7. Second, a notable proportion of responses occurred in patients who were considered to be resistant to cisplatin (disease progression $\leq$ 6 mo after cisplatin treatment). Responses occurred in six of 25 cisplatin-resistant patients and in six of 15 patients who did not show "clear refractoriness" to cisplatin (disease progression $>$ 6 mo after cisplatin treatment). Finally, the doses of taxol given to most patients (22% at 110 mg/m^2; 48% at 135 mg/m^2; and 22% at 170 mg/m^2) were significantly lower, because of limited hematopoietic tolerance, than doses previously demonstrated to be safe in minimally pretreated or untreated patients (200–250 mg/m^2).

A clear dose–response relationship was not defined because of the extent of previous therapy and subsequent inability to administer high doses. However, the demonstration of such a relationship in future studies involving higher doses and less heavily pretreated patients may serve as the rationale for the use of colony-stimulating factors with taxol or combinations of taxol and cisplatin to permit treatment with higher taxol doses. Even so, severe mucositis at doses of taxol administered as a single agent that are only slightly greater than doses that induce severe neutropenia may preclude any benefits of colony-stimulating factors with respect to dose intensity (*80*).

The Gynecology Oncology Group (GOG) and investigators at the Albert Einstein School of Medicine are completing confirmatory studies in advanced ovarian cancer, and preliminary results confirm activity of taxol in advanced and refractory ovarian carcinomas (*106,107*). The GOG has reported an overall response rate (partial responses + complete responses) of 37% in patients who previously received cisplatin-based therapies. This rate includes responses in seven of 14 patients (50%) who were not resistant to cisplatin (disease progression $>$6 mo after cisplatin) and eight of 24 (33%) with cisplatin resistance (progression $\leq$6 mo after cisplatin (*107*). Investigators at the Albert

Einstein College of Medicine recently reported on six responses (one pathological complete response and five partial responses) in 30 assessable, previously treated patients. Four of the six responses occurred in patients with cisplatin-resistant disease (*106*) (Einzig AI: personal communication).

Toxic Effects

Hematologic toxic effects. Neutropenia, the principal dose-limiting toxic effect of taxol, has not been schedule dependent (*92–105*). The onset of neutropenia has usually occurred by day 8, and nadir neutrophil counts have generally occurred on days 8–11, with rapid recovery by days 15–21. Episodes of fever or sepsis associated with severe neutropenia have been infrequent, which is probably because of the short duration of taxol-induced neutropenia. In fact, McGuire et al. (*105*) have reported that 213 of 281 courses of taxol in heavily pretreated patients with ovarian cancer were associated with grades 3 or 4 neutropenia (neutrophil counts <1,000 cells/ μL). However, only 14 patients (21 of 281 courses) required one or more hospitalizations for fever associated with neutropenia.

There is also little evidence that neutropenia is cumulative, and neutrophil count nadirs have generally remained unchanged during successive courses, indicating that taxol may not be irreversibly toxic to hematopoietic stem cells. The major clinical risk factor for neutropenia appears to be the extent of prior myelotoxic chemotherapy and/or irradiation (*103*). McGuire et al. (*105*) reported that severe (grade 3 or 4) neutropenia of brief duration was consistently observed in the majority of heavily pretreated ovarian cancer patients who received 24-hour infusions of taxol at doses of 110–135 mg/m^2. These doses had previously produced negligible neutropenia in minimally pretreated patients.

To evaluate the influence of pharmacologic disposition on the severity of myelosuppression, Longnecker et al. (*108*) correlated relevant pharmacokinetic parameters with hematologic parameters in a phase I study. The investigators demonstrated a correlation between the area under concentration-versus-time curves and white blood cell count nadirs, changes in the absolute white blood cell count, and percent decreases in white blood cell counts. Significant anemia and thrombocytopenia have rarely been observed, even in heavily pretreated patients with severe neutropenia. Interestingly, taxol has been demonstrated to produce characteristic changes in platelet microtubules in vitro, but it does not perturb platelet biochemistry or structure (*109*).

Hypersensitivity. A major concern during early clinical studies of taxol was the occurrence of hypersensitivity reactions. Weiss et al. (*110*) recently reviewed the characteristics of 32 patients judged to have had true hypersensitivity reactions at some time during 1983–1988. Of those 32, 27 had definite manifestations of type I hypersensitivity reactions, including hypotension, dyspnea with bronchospasm, and urticaria that appeared to be mediated by the release of histamine. Other less definite evidence of type I reactions was present, such as abdominal and extremity pain, angioedema, and diaphoresis. Five other patients had manifestations that suggested hypersensitivity reactions; these included hypotension, generalized erythema, pruritus without skin lesions, and dyspnea without bronchospasm.

Fifty-three percent of the hypersensitivity reactions occurred within 2–3 minutes after taxol treatment was initiated, when some patients had received only 2–3 mg of drug; 78% of the patients had symptoms within 10 minutes of starting taxol. The other reactions began 30–90 minutes after treatment was started, but two patients developed hypersensitivity reactions 3 and 12 hours after completing taxol treatment. One patient had a fatal reaction that was characterized by the rapid development of hypotension and asystole, but all other patients recovered fully without any adverse consequences, after various treatments, including administration of fluids, antihistamines, vasopressors, corticosteroids, and aminophylline. Seven patients, including five who received prophylactic anti-allergic drugs, received subsequent courses of taxol without further problems.

Taxol-induced hypersensitivity reactions do not seem to be caused solely by IgE directed against taxol or its cremophor vehicle, since most episodes have occurred during the first treatment. In addition, prior sensitization to an antigen is generally thought to be necessary for the development of an IgE response. Weiss et al. (*110*) reported that hypersensitivity reactions occurred with the first dose of taxol in 18 patients, the second dose in 13 patients, and the sixth dose in one patient. It seems more plausible that these hypersensitivity reactions are principally nonimmunologically mediated by the direct release of histamine or other vasoactive substances from mast cells and basophils. This mechanism is analogous to the presumed mechanism for hypersensitivity reactions caused by iodinated radiocontrast dyes (*111,112*).

It is also unclear whether taxol itself or its cremophor vehicle is principally responsible for hypersensitivity reactions. As previously discussed, cremophor has been implicated in similar hypersensitivity reactions in dogs. The reactions have been attributed to the release of histamine (*89*). In addition, other drugs formulated in cremophor, such as cyclosporine, teniposide, vitamin K, and didemnin B have been associated with similar hypersensitivity reactions. However, the clinical formulation of therapeutic taxol doses requires the greatest amount of cremophor relative to these other agents (*113*).

Initially, hypersensitivity reactions appeared to be indirectly related to the duration of the taxol infusion, and therefore indirectly related to the concentrations of taxol and cremophor in the infusate. However, Weiss et al. (*110*) reported that 11 of 32 reactions occurred with 24-hour infusions. In fact, the incidence rates of hypersensitivity reactions in this series were 16%, 13%, and 7% with 3-, 6-, and 24-hour infusions, respectively, but the majority of patients received taxol over 24 hours after prophylactic anti-allergic medications.

After hypersensitivity reactions were recognized as a significant problem, investigators and the NCI recommended that subsequent phase I and II studies should utilize 24-hour infusions and prophylactic anti-allergic premedications. These premedications included dexamethasone, the H_1-histamine antagonist diphenhydramine, and either one of the H_2-histamine antagonists, cimetidine or ranitidine. This recommendation was, in part, based on the successful prophylaxis of hypersensitivity reactions by similar regimens in patients who were allergic to iodinated radiocontrast agents (*114–116*).

Initially, the premedications appeared to reduce the incidence and severity of hypersensitivity reactions (*93*), but it was later recognized that they were not fully protective. In fact, 11 of the 27 definite type I hypersensitivity reactions in the series of Weiss

et al. occurred despite these prophylactic measures (*110*). However, only one hypersensitivity reaction was reported in each of three later studies that used anti-allergic premedications. These later trials involved 28 courses in 17 patients with acute leukemia and 281 courses in 47 ovarian cancer patients (*100,105*); one hypersensitivity reaction has also occurred in a study involving 150 courses of taxol and cisplatin administered to 38 patients (*100*) (Rowinsky EK: unpublished data). Although the true incidence of hypersensitivity reactions and the relative benefits of specific administration schedules and premedication regimens are not clearly known, the NCI has recommended that subsequent studies of taxol utilize 24-hour infusions. In addition, prophylactic anti-allergic premedications have been recommended, with the following drugs and dosages:

(*a*) dexamethasone—20 mg injected orally or intravenously 14 and 7 hours before taxol treatment.
(*b*) diphenhydramine—50 mg injected intravenously 30 minutes before taxol treatment; and
(*c*) cimetidine or ranitidine—300 mg or 50 mg, respectively, injected intravenously 30 minutes before taxol treatment.

Gastrointestinal toxic effects. Although mucositis was rarely observed in phase I studies of solid tumors (*82–88*), it was the major nonhematologic dose-limiting toxicity in a phase I study that evaluated high taxol doses in leukemia patients (*80*). In this study, severe mucositis precluded dose escalation above 390 mg/m^2. At the recommended phase II dose for leukemia patients, 315 mg/m^2, severe mucositis occasionally occurred only during second or third courses, which suggested that this toxic effect was, in part, cumulative. Mucositis was generally more severe during subsequent courses if the later treatment was administered 15 days or less after the previous treatment, indicating that subclinical mucosal repair was incomplete even until 15 days after treatment with taxol at doses of 315 mg/m^2 or more. Although all patients in the trial received extensive previous chemotherapy, prior treatment with other well-known mucosal toxins, such as etoposide or busulfan, was not associated with more severe mucositis.

Taxol-induced mucositis has been characterized by diffuse ulcerations of the lips, oral cavity, and pharynx. Dysphagia and pain reflecting esophageal involvement have also been common. Postmortem examinations have revealed mucosal ulcerations in the oropharynx, esophagus, and intestines without evidence of viral, fungal, or bacterial mucosal invasion (*117*). Ultrastructurally, an accumulation of epidermal cells with taxol-induced asters has been evident in ulcerated mucosa, indicating cell cycle arrest in mitosis (*117*). Severe mucositis has usually been associated with severe pain, and continuous infusions of narcotics have often been required. Oropharyngeal ulcerations have been noted 3–7 days after treatment initiation; they have resolved within 5–7 days.

Taxol-related gastrointestinal toxic effects have also included nausea, vomiting, and diarrhea, which have generally been infrequent and modest in severity.

Neurological toxic effects. Taxol has been known to inhibit neurite growth and induce prominent morphological effects, such as microtubule bundles in neurons, in satellite cells, and in Schwann cells in organotypic dorsal root ganglion cultures (*118–123*). Taxol also inhibits the regenerative response of axons and Schwann cells after nerve-crush injuries (*124,125*). However, taxol does not affect the release of the neurotransmitter acetylcholine in synaptosomes or neurite formation in neuro-2a neuroblastoma cells (*126,127*). Because of these experimental data and the importance of microtubules in axonal transport and other vital neuron functions, neurotoxicity might have been expected to be a principal toxic effect of taxol in animal toxicology studies. Indeed, neurotoxicity has been a major toxic effect of other antimicrotubule drugs. However, neurotoxicity was not apparent in studies in mice, rats, and dogs; this finding may have been caused by the limitations of those models in detecting this effect (*81*).

Neurotoxicity has not been observed in phase I studies of taxol administered on single-dose, multiple-dose, or 3-hour infusional schedules (*94–97*). Although peripheral neurotoxicity has frequently occurred with 6- and 24-hour infusions (*80,92–95*), it has been rare at doses below 170 mg/m^2. In general, the incidence and severity of peripheral neurotoxicity has been dose related. In a phase I study of taxol administered over 24 hours to patients with solid tumors, peripheral neurotoxicity, as well as myelosuppression, precluded dose escalation above 275 mg/m^2 (*95*). However, in a study of leukemias, all patients treated with doses of 315–390 mg/m^2 over 24 hours complained of mild to moderate paresthesias and numbness, but neurotoxicity was not the principal dose-limiting, nonhematologic toxic effect (*80*).

Taxol-related peripheral neurotoxicity has been principally characterized by neurosensory manifestations. The most common symptoms have been numbness and paresthesias in a glove-and-stocking distribution. Perioral numbness has also been described. Symptoms have begun as early as 24–72 hours after treatment with higher doses ($\geq$250 mg/m^2), but they have also occurred after multiple courses at lower doses. Neurotoxicity has been cumulative and has progressively worsened after multiple courses at higher doses. Initially, most patients have complained of burning pain, particularly in the feet. The pain has often been associated with hyperesthesias. Neurological examinations have revealed distal sensory loss to large (proprioception, vibration) and small (pin prick, temperature) fiber modalities (*80,92–95,105,126*). Lost or decreased distal deep tendon reflexes have also been common in patients with neuropathic complaints.

Lipton et al. (*128*) have reported electrophysiological findings in several symptomatic patients, including decreased nerve conduction velocities in sensory nerves, with relative sparing of motor nerves. Significant elevations in vibratory and thermal threshold elevations have also been noted (*94,95,128*). These electrophysiological data have supported both axonal degeneration and demyelination as mechanisms for taxol-induced neurotoxicity (*128*).

Sensory symptoms have usually improved or resolved within several months after discontinuation of taxol (*80,92–95*). Areflexia has also resolved completely (*94*). In one report, a sural nerve biopsy performed on a patient with severe neurotoxic symptoms that did not resolve completely after treatment revealed no disarray or aggregation of microtubules in axons or Schwann cells (*94*). Instead, it showed thinly myelinated axons that suggested remyelination. Amitriptyline has been found by some investigators, but not others, to be useful in ameliorating residual neuropathic symptoms (*80,95*). Interestingly, nerve

growth factor, a neuronotrophic factor required for maintenance of sympathetic and dorsal root ganglion cells in culture, has been shown to attenuate the microtubule-disrupting effects of taxol in organotypic cultures (*129,130*).

Although taxol-induced motor disturbances have generally been absent or mild, severe motor dysfunction has also been reported. Transient paralytic ileus and severe generalized weakness that briefly prevented ambulation have been described in two diabetic patients after 24-hour infusions of taxol at 250 mg/m^2 (*94*). In addition, mild motor dysfunction in the lower extremities has been described in a patient with refractory leukemia who received three 24-hour infusions of taxol at doses of 200–315 mg/m^2 (*80*).

Risk factors for the development of peripheral neurotoxicity, such as previous therapy with other neurotoxic chemotherapeutics, were examined in one phase I study (*93*). In that study, 18 patients were treated with potentially neurotoxic taxol doses of 170–265 mg/m^2 over 6 hours. Of the 11 patients in this group who previously received cisplatin or vinca alkaloids, nine developed symptomatic neurotoxicity. Seven patients had received no prior neurotoxic therapy, but five still developed neuropathic effects. McGuire et al. (*105*) also reported that one to 20 courses of taxol induced either no neuropathic symptoms or mild neuropathic symptoms in the majority of ovarian cancer patients who had been heavily pretreated with cisplatin-based chemotherapy. However, the extent of prior chemotherapy and neutropenia limited taxol doses during most courses in that trial to 110–170 mg/m^2.

In addition, neurotoxicity has been evaluated in a prospective, ongoing phase I study of taxol and cisplatin (*100*). Investigators have used rigorous sequential neurological and neurometric examinations. In that study, neurotoxic symptoms have occurred infrequently, even after multiple courses of cisplatin (50–75 mg/m^2) and taxol (110–200 mg/m^2) (Rowinsky EK: unpublished data). Thus far, patients with a history of substantial alcohol use have appeared to be more predisposed to the development of neurosensory toxic effects due to cisplatin and taxol.

Transient myalgias and arthralgias have also been observed with higher taxol doses and prolonged infusion durations (*80,93,105*). These symptoms have occurred 2–3 days after treatment and resolved within 5 days. Severe myalgias and arthralgias that required narcotics for palliation have also been observed with higher doses (315–390 mg/m^2) (*80*). Shoulder and paraspinal muscles have usually been involved, and patients have typically complained of arthralgias in the large joints of the arms and legs. Concurrent signs of inflammation and elevations in levels of muscle enzymes, such as creatine phosphokinase, have not been noted. A grand mal seizure has also been observed during a taxol infusion administered to a 34-year-old woman with ovarian carcinoma (*105*). Computerized tomographic and magnetic resonance scans of the brain, examination of cerebrospinal fluid, and electroencephalogram showed no abnormality, but seizures recurred after taxol treatment was restarted.

Cardiotoxicity. Transient asymptomatic bradycardia has frequently been noted during taxol infusions in patients without cardiac risk factors. McGuire et al. (*105*) reported asymptomatic bradycardia during one or more courses in 29% of ovarian cancer patients. Therefore, asymptomatic bradycardia alone is probably not an indication for discontinuing taxol. However, two patients developed more significant bradyarrhythmias related to taxol, including a progressive atrioventricular block that culminated in third-degree block and 7 seconds of asystole in one patient (*105*). Although the patient remained asymptomatic, a pacemaker was inserted and subsequent taxol infusions were associated with capture of paced beats when the sinus rate fell below the demand rate. This rhythm reverted to an unpaced rhythm after each taxol infusion. A second patient developed Wenckebach syndrome during each of several courses; the syndrome resolved within hours after each infusion. Although it is uncertain whether taxol or its cremophor vehicle are directly responsible for bradyarrhythmias, taxol has been suspected because other agents formulated in cremophor have not been associated with similar arrhythmias.

Ventricular tachycardia has recently been observed in four patients who were monitored during taxol infusions in a phase I study of cisplatin and taxol (*100*) (Rowinsky EK: unpublished data). Ten seconds of ventricular tachycardia, resulting in the abrupt discontinuation of taxol, occurred in one asymptomatic patient with non-small cell lung carcinoma. Considerable ventricular ectopy was observed after administration of taxol; it ceased after lidocaine treatment was initiated. The patient's malignancy progressed, but taxol was not readministered. The three other patients had limited 4–8 beat episodes of ventricular tachycardia that did not recur either during the remainder of the infusion or during subsequent treatments. Holter monitor readings of outpatients, which were recorded in three of the four patients to assess intrinsic cardiac rhythms, were normal in two patients and revealed similar ectopy in the third patient.

Several patients have also complained of atypical chest pains during taxol infusions. These pains may actually be manifestations of hypersensitivity reactions (*80,83*). However, a fatal myocardial infarction that was confirmed on postmortem examination and was not preceded by an arrhythmia has also occurred during an infusion of taxol administered to a patient with atherosclerotic cardiovascular disease (Rowinsky EK: unpublished data). Although these cardiac events have occurred infrequently, they are of significant concern in light of anticipated broad phase II testing.

Other toxic effects. Alopecia has been observed in almost all patients treated with taxol at doses of 135 mg/m^2 or more. Like alopecia caused by other antineoplastic agents, taxol-induced alopecia has been reversible, but it has been unique in other respects. The loss of scalp hair, which has generally occurred between days 14–21, has been sudden and complete, often occurring in a single day. In addition, patients have often experienced a loss of all body hair including axillary pubic and extremity hair, eyelashes, and eyebrows.

Other drug- and/or cremophor-related effects have included local venous toxic effects, with erythema, tenderness, and discomfort along the course of an injected vein and cellulitis in areas of dermal extravasation. Fatigue, headaches, taste perversions, and minor elevations in hepatic and renal functions have also been noted infrequently in patients with progressive cancer. In addition, taxol has also produced significant elevations in serum triglyceride levels (*99*).

Clinical Pharmacology

Highly sensitive and specific reverse-phase, high-performance liquid chromatographic assays capable of detecting taxol concen-

Table 2. Taxol pharmacokinetic parameters

Schedule (Institution)* (Ref. No.)	Model	$T_{½}\alpha$ (hr)	$T_{½}\beta$ (hr)	Cl† (mL/min per m^2)	C_p†		VD_{ss}† (L/m^2)	MRT† (hr)	Urine (% dose)
					μmol/L	Dose (mg/m^2)			
6-hr infusion (Einstein) (*94*)	Biphasic	0.32	8.6	100	3.2–8.1	175–275	55	8.6	5.2
24-hr infusion (Einstein) (*95*)	Biphasic	0.27	3.9	993	0.6–0.94	200–275	182	19.9	1.4
1- to 6-hr infusion × 5 days (Wisconsin) (*97*)	Biphasic	—	1.3	833	0.06–0.37	15–40	81	—	6.6
1- to 6-hr infusion (Hopkins) (*108*)	Biphasic	0.27	6.4	253	1.3–13.0	60–265	67	5.6	5.9
24-hr infusion (Hopkins) (*80*)	—	—	—	—	1.6–3.5	250–390	—	—	—
6-hr infusion UTSA (*92*)	Biphasic	—	4.8	300	2.3–4.6	175–275	167	11.8	—
Mean	—	0.29	5.0	496	—	—	110	11.5	4.8
SD		0.03	2.7	392			59	6.2	2.3

*Einstein = Albert Einstein College of Medicine, New York, NY; Wisconsin = University of Wisconsin-Madison; Hopkins = Johns Hopkins University, Baltimore, Md; UTSA = The University of Texas, San Antonio.
†Cl = systemic clearance; C_p = peak plasma concentration; VD_{ss} = volume of distribution at steady state; MRT = mean resonance time.

trations as low as 50 nmol/L have been developed, and the pharmacology of taxol in humans has been studied during six phase I trials (*80,92,94–96,108*). Table 2 summarizes several pertinent pharmacokinetic parameters. Taxol's disposition in plasma has been characterized best by a biexponential model. Although there has been wide variability in distribution (alpha) and elimination (beta) half-lives among patients, evidence for nonlinearity or dose dependency has not yet been found. Alpha and beta half-lives have ranged from 0.27–0.32 hours (mean, 0.29) and 1.3–8.6 hours (mean, 5.0), respectively.

Despite extensive binding to plasma proteins (95%–98%), as determined by both ultracentrifugation and equilibrium dialysis, taxol is readily eliminated from plasma, which is consistent with limited preclinical data (*84,85,87,99*). Mean values for steady-state volumes of distribution have been large and have ranged from 55 to 183 L/m^2 (mean, 110). Systemic clearances have ranged from 100 to 993 mL/min per m^2 (mean, 496), but the principal mechanisms of systemic clearance have not been clarified.

Total urinary excretion has been insignificant (range, 1.4%–6.6%, mean, 4.8%), which indicates that renal clearance contributes minimally to systemic clearance. This fact also suggests that metabolism, biliary excretion, and/or extensive tissue binding are responsible for the bulk of systemic clearance. In fact, high taxol concentrations and an unidentified metabolite have recently been measured in the bile of two patients with biliary catheters (Rowinsky EK: unpublished data). Metabolites in blood and urine have not been identified, but minor (<5%) spontaneous conversion of taxol to 7-epitaxol has been demonstrated to occur in normal saline solutions at 37 °C after 48 hours and also in tissue culture (*92,131*). To date, taxol has been measured in the ascites of one patient 7 hours postinfusion and maintained for at least 12 hours at concentrations that were 40% of concurrent plasma levels (*94*). However, taxol was not detected postinfusion in the cerebrospinal fluid of leukemia patients (*78*). More importantly, the range of peak plasma taxol levels achieved on all schedules has been demonstrated to be capable of inducing significant biologic and cytotoxic effects in clinical specimens in vitro (*35*).

Future Directions

The responses observed with taxol in advanced and cisplatin-refractory ovarian carcinomas have generated considerable enthusiasm. In fact, these results are reminiscent of early studies with cisplatin in the 1970s, in which response rates of approximately 30% occurred in patients with drug-refractory ovarian cancer and 50%–60% in untreated patients (*132,133*). Defining the role of taxol in first- and second-line treatment for untreated patients with ovarian cancer, as well as patients with refractory and recurrent ovarian cancer, has become a goal in the development of this agent.

In addition to pivotal trials in ovarian cancer, the current development program is directed toward evaluating the spectrum of taxol's activity in other disease sites. However, given the current constraints on the supply of taxol, continued clinical investigation remains limited. Current supplies must be used judiciously; therefore, ongoing and planned trials in specific disease sites were selected after analysis of (*a*) preclinical antitumor activity data, (*b*) responses in phase I and limited phase II trials, and (*c*) consideration of clinical studies that would explore activity in other platinum-sensitive tumors and common adult malignancies.

Several phase II trials (non-small cell lung carcinoma and

gastric, breast, colon, and cervical carcinomas) have recently opened to patient accrual. Further phase II testing is planned for head and neck, small cell lung, and prostate carcinomas and for carcinomas at other sites, as supplies permit. In addition, a phase III study of taxol and cisplatin versus cisplatin and cyclophosphamide in untreated patients with suboptimal ovarian carcinoma has opened to patient accrual. The question of dose intensification for treatment of solid tumors will be addressed in phase I trials of colony-stimulating factors and taxol, both as single agents and in combination with cisplatin. Phase I testing is also planned in the pediatric patient population. A more comprehensive analysis of taxol's antitumor activity at other disease sites will be undertaken as additional supplies become available.

However, encouraging results in ongoing and subsequent phase II and III studies of taxol will be to no avail without a feasible long-term solution to the problem of supplying adequate quantities of this scarce natural compound or the identification of suitable analogues for widespread clinical use. The NCI, in collaboration with industry, will attempt to resolve the formidable technical problems that threaten future supplies. Currently, 20,000 lb of bark from 2,000–3,000 trees are required to produce 1 kg of taxol. Although taxol has been identified "as possibly the number one target today of synthetic organic chemists (*134,135*)," the total synthesis of this complex molecule remains a difficult task and an as yet unattained and possibly unrealistic goal.

One possible option may be the hemisynthesis of taxol or active analogues from more abundant natural compounds such as baccatin III, one of the less complex diterpenoids that is derived from the needles of *Taxus baccata*. Both taxol and a related compound, taxotere (RP 56976, NSC 628503), which appears to be even more active than taxol against several murine tumors, have already been synthesized from baccatin III (*136,137*). Another avenue of exploration is the extraction of taxol and related analogues from needles harvested from yew bushes (*134*). Bushes would remain intact and serve as a renewable source of yew needles. In addition, improvements in plant cell culture techniques have permitted investigators to grow taxol-producing plant cells in vitro and provide a potentially viable solution to the current supply problem (*138*).

Taxol possesses a unique mechanism of cytotoxic activity. For this reason, in addition to preclinical antitumor activity and responses observed in early clinical trials, the expenditure of resources for taxol's further clinical development appears to be warranted at this time.

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